



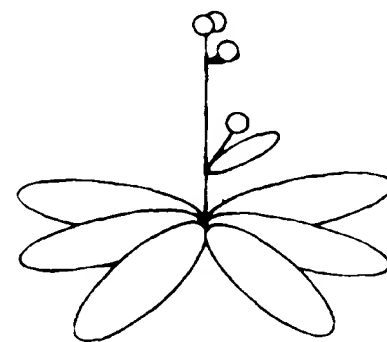
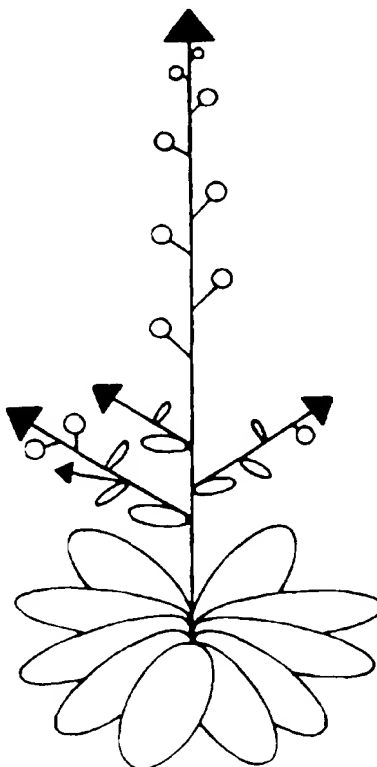
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(57) Abstract

The *cen* gene of *Antirrhinum* has been cloned, also homologues from *Arabidopsis* (*ft1*) and rice. Flowering characteristics of transgenic plants, especially switching of apical meristem to a floral fate and the timing of flowering, may be manipulated by regulating gene expression. The promoter of the *cen* gene may be used to drive tissue-specific expression, specifically in the apical meristem of plants.



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FLOWERING GENES

The present invention relates to genetic control of flowering and is based on the cloning of the *cen* gene of *Antirrhinum* and the *tfl1* gene of *Arabidopsis*.

5 There are three main types of meristem involved in ariel plant development; vegetative, inflorescence and floral. The apical meristem in many species, such as *Antirrhinum majus*, first undergoes a vegetative phase whereby cells set aside from the apex become
10 leaf primordia with an axillary vegetative meristem (Coen, 1991). Upon floral induction, the apical meristem is converted to an inflorescence meristem. The traits commonly associated with the inflorescence are the modification of leaf organs and a change in
15 internode length. The inflorescence of *Antirrhinum* is a raceme or spike, with the apical meristem growing indeterminately. Floral meristem arise in the axils of modified leaves and are determinate, producing four whorls or rings of floral organ primordia. Thus the
20 apical meristem goes through two distinct identities, vegetative and then inflorescence. In species which produce terminal flowers, the apical meristem is determinate and eventually adopts a third identity, that of a floral meristem. A key developmental
25 question has been to understand how the identity of the apical meristem is controlled.

The *centroradialis (cen)* mutant of *Antirrhinum* was first described in Gatersleben, Germany (Kuckuck

and Schick, 1930; Stubbe, 1966). The *cen* mutant produces a number of axillary flowers before the apical meristem is converted to a floral meristem. Thus in *cen* plants, the apical meristem goes through
5 three distinct identities; vegetative, inflorescence and then floral. The wild-type role of *cen* is therefore to prevent the apical meristem from switching to a floral fate.

Cen mutants of *Antirrhinum* may differ from wild
10 type in several respects. Mutants produce a terminal flower, converting the inflorescence from indeterminate to determinate. Consequently, the architecture is changed to a shorter, more bushy plant, as shoots cannot grow indefinitely. About 10
15 axillary flowers are made below the terminal flower. The terminal floral meristem is developmentally more advanced than the axillary flowers below it. Unlike axillary flowers, organ numbers and their arrangement (phyllotaxy) are very variable in terminal flowers.
20 The terminal flower is usually radially symmetrical, with all petals resembling the ventral (lowest) petal of axillary flowers.

A similar mutant to *cen*, *terminal flower1* (*tfl1*), has been described in *Arabidopsis* (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). In addition to
25 affecting meristem identity, *tfl1* mutations also result in early flowering. Therefore, the normal role of the *tfl1* gene is to inhibit flowering as well as

preventing the apical meristem from switching to a floral fate.

In *Arabidopsis*, *tfl1* mutants have two key features distinguishing from wild type: bolting early and the apical meristem eventually acquiring floral identity, leading to the production of a terminal flower (Figure 1). Typically, about half the normal number of rosette leaves are produced before bolting and about 1-5 peripheral flowers are made before the inflorescence apical meristem finally acquires floral identity. The structure of the terminal flower is often different to the wild-type. Wild-type flowers consist of 4 whorls of organs; 4 sepals outermost, 4 petals, 6 stamens and a central whorl of 2 unlimited carpels. In the terminal flower of *tfl1* mutants in *Arabidopsis*, numbers of organs often vary and they may arise in a spiral, unlike the whorled arrangement of wild-type. Mosaic organs, composed of two types of floral organ, can also be found. All of these phenotypic effects, except for a marked change in flowering time, are also seen in *cen* mutants of *Antirrhinum*.

Both these genes therefore play key roles in apical meristem identity.

To delineate the action of *cen* and the molecular pathway by which it acts, a transposon-mutagenesis programme was set up to isolate the gene. In 1992, three new alleles of *cen* (*cen*-663, *cen*-665 and *cen*-

666) were successfully isolated and a transposon linked to the *cen* phenotype in one allele was identified. Early in 1994, the flanking DNA of this transposon insertion was used to reveal that the *cen* locus had been cloned, allowing isolation of the *cen* cDNA and characterisation of its expression. CEN has similarly to a class of animal lipid-binding proteins and is expressed in the shoot apex.

The present invention is based on cloning of the *cen* gene from *Antirrhinum* and a homologue from *Arabidopsis*, *tfl1*. See also Bradley et al., *Nature* 1996, Vol. 379, 791-797 (*cen*) and Bradley, Carpenter and Coen, "Conserved control of inflorescence architecture in *Arabidopsis* and *Antirrhinum*", submitted.

According to an aspect of the present invention there is provided a nucleic acid isolate comprising a nucleotide sequence encoding a polypeptide with *cen*, *tfl1* or indeterminacy function. Those skilled in the art will appreciate that the terms "*cen* function", "*tfl1* function" and "indeterminacy function" refer to the ability to influence the timing of flowering and/or the prevention of meristems switching to a floral fate phenotypically like the respective *cen* or *tfl1* gene of *Antirrhinum* or *Arabidopsis*.

"Indeterminacy function" refers to ability to keep the meristem growth indeterminant. Certain embodiments of the present invention may have ability to

complement a *cen* or *tfl1* mutation in *Antirrhinum* or *Arabidopsis*.

Nucleic acid according to various aspects of the present invention may have the sequence of a *cen* or
5 *tfl1* gene or be a mutant, variant, derivative or allele of the sequence provided. Preferred mutants, variants, derivatives and alleles are those which encode a product (nucleic acid molecule or polypeptide) which retains a functional characteristic
10 of the product encoded by the wild-type gene, especially, as for *cen*, the ability to inhibit apical meristem from switching to a floral fate and/or, as for *tfl1*, the additional ability to inhibit/delay flowering. Other preferred mutants, variants,
15 derivatives and alleles encode a product which promote flowering compared to wild-type or a gene with the sequence provided and/or promote switching of apical meristems to a floral fate. Changes to a sequence, to produce a mutant, variant or derivative, may be by one
20 or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, which may lead to the addition, insertion, deletion or substitution of one or more amino acids in an encoded polypeptide product. Of course, changes to
25 the nucleic acid which make no difference to the encoded amino acid sequence are included.

In a preferred embodiment of the present invention a nucleic acid molecule comprises a

nucleotide sequence which encodes an amino acid sequence shown in Figure 4(a). The nucleotide sequence may comprise an encoding sequence shown in Figure 4(a) or may be a mutant, variant, derivative or
5 allele thereof encoding the same amino acid sequence.

In a further embodiment, a preferred nucleic acid molecule according to the present invention comprises a nucleotide sequence encoding an amino acid sequence shown in Figure 6(a) or may be a mutant, variant,
10 derivative or allele thereof encoding the same amino acid sequence.

Sequences comprising changes to or differences from the sequences shown in the figures may also be employed in the present invention, as discussed
15 herein.

The present invention also provides a vector which comprises nucleic acid with any of the provided sequences, preferably a vector from which a product polypeptide or nucleic acid molecule encoded by the
20 nucleic acid sequence can be expressed. The vector is preferably suitable for transformation into a plant cell. The invention further encompasses a host cell transformed with such a vector, especially a plant cell. Thus, a host cell, such as a plant cell,
25 comprising nucleic acid according to the present invention is provided. Within the cell, the nucleic acid may be incorporated within the chromosome. There may be more than one heterologous nucleotide sequence

per haploid genome. This, for example, enables increased expression of the gene product compared with endogenous levels, as discussed below.

A vector comprising nucleic acid according to the present invention need not include a promoter, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

Nucleic acid molecules and vectors according to the present invention may be provided isolated and/or from their natural environment, in substantially pure or homogeneous form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may comprise cDNA, RNA, genomic DNA and may be wholly or partially synthetic. The term "isolate" may encompass all these possibilities.

The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and methods of making the expression product by expression from encoding nucleic acid therefor under suitable conditions in suitable host cells. Those skilled in the art are well able to construct vectors and design protocols for expression and recovery of products of recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory

sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, 5 *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Transformation procedures depend on the host used, but are well known. Many known techniques and protocols for manipulation of nucleic acid, for 10 example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. 15 eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

Purified protein, or a fragment, mutant or variant thereof, e.g. produced recombinantly by 20 expression from encoding nucleic acid therefor, may be used to raise antibodies employing techniques which are standard in the art. Antibodies and polypeptides comprising antigen-binding fragments of antibodies may be used in identifying homologues from other species 25 as discussed further below.

Methods of producing antibodies include immunising a mammal (eg mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment

thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and might be screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, Nature 357: 80-82). Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal, antibodies with appropriate binding specificity may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, eg using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibodies raised to a polypeptide or peptide can be used in the identification and/or isolation of homologous polypeptides, and then the encoding genes. Thus, the present invention provides a method of identifying or isolating a polypeptide with the desired function (in accordance with embodiments disclosed herein), comprising screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind a CEN or Tfl1 polypeptide or fragment or variant thereof or preferably has binding specificity for such

a polypeptide, such as having the amino acid sequence shown in Figure 4 or Figure 6. Specific binding members such as antibodies and polypeptides comprising antigen binding domains of antibodies that bind and
5 are preferably specific for such a polypeptide or mutant, variant or derivative thereof represent further aspects of the present invention, as do their use and methods which employ them.

Candidate polypeptides for screening may for
10 instance be the products of an expression library created using nucleic acid derived from an plant of interest, or may be the product of a purification process from a natural source.

A polypeptide found to bind the antibody may be
15 isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the polypeptide either wholly or partially (for instance a fragment of the polypeptide may be sequenced). Amino acid sequence information may be
20 used in obtaining nucleic acid encoding the polypeptide, for instance by designing one or more oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridisation to candidate nucleic acid, or by
25 searching computer sequence databases.

The nucleotide sequence information provided herein or any part thereof may be used in a data-base search to find homologous sequences, expression

products of which can be tested for ability to influence a flowering characteristic of a plant. By sequencing homologues, studying their expression patterns and examining the effect of altering their expression, genes carrying out a similar function are obtainable.

A further aspect of the present invention provides a method of identifying and cloning *cen* homologues from plant species other than *Antirrhinum majus* which method employs a nucleotide sequence derived from any shown in the figures. The nucleotide sequence information provided herein, or any part thereof, may be used in a data-base search to find homologous sequences, expression products of which can be tested for ability to influence a plant meristem and/or other flowering characteristic. These may have *cen* or *tfl1* function or the ability to complement a respective mutant phenotype. In a preferred embodiment the sequence employed is one shared by the *cen* and *tfl1* genes provided herein. Nucleic acid libraries may be screened using techniques well known to those skilled in the art and homologous sequences thereby identified then tested.

For instance, such a method may employ an oligonucleotide or oligonucleotides which comprises or comprise a sequence or sequences that are conserved between the sequences of Figures 4 and 6 to search for homologues. Thus, a method of obtaining nucleic acid

whose expression is able to influence a flowering characteristic of a plant is provided, comprising hybridisation of an oligonucleotide or a nucleic acid molecule comprising such an oligonucleotide to
5 target/candidate nucleic acid. Target or candidate nucleic acid may, for example, comprise a genomic or cDNA library obtainable from an organism known to contain or suspected of containing such nucleic acid. Successful hybridisation may be identified and
10 target/candidate nucleic acid isolated for further investigation and/or use.

Hybridisation may involve probing nucleic acid and identifying positive hybridisation under suitably stringent conditions (in accordance with known
15 techniques) and/or use of oligonucleotides as primers in a method of nucleic acid amplification, such as PCR. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations
20 identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

As an alternative to probing, though still
25 employing nucleic acid hybridisation, oligonucleotides designed to amplify DNA sequences may be used in PCR reactions or other methods involving amplification of nucleic acid, using routine procedures. See for

instance "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, 1990, Academic Press, New York.

Preferred amino acid sequences suitable for use
5 in the design of probes or PCR primers are sequences conserved (completely, substantially or partly) between at least two polypeptides able to influence a flowering characteristic, particularly the switching of apical meristem to a floral fate, e.g. with the
10 amino acid sequences of Figures 4 and 6 herein.

On the basis of amino acid sequence information oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the
15 organism from the candidate nucleic acid is derived.

Preferably an oligonucleotide in accordance with the invention, e.g. for use in nucleic acid amplification, has about 10 or fewer codons (e.g. 6, 7 or 8), i.e. is about 30 or fewer nucleotides in length
20 (e.g. 18, 21 or 24).

Assessment of whether or not such a PCR product corresponds to resistance genes may be conducted in various ways. A PCR band from such a reaction might contain a complex mix of products. Individual
25 products may be cloned and each one individually screened. It may be analysed by transformation to assess function on introduction into a plant of interest.

The present invention also extends to nucleic acid encoding a *cen* or *tfl1* homologue obtained using a nucleotide sequence derived from the sequence information (amino acid and/or nucleotide) presented
5 in the figures.

Thus, included within the scope of the present invention are nucleic acid molecules which encode amino acid sequences which are homologues of *cen* of *Antirrhinum* or *tfl1* of *Arabidopsis*. Homology may be
10 at the nucleotide sequence and/or amino acid sequence level. Preferably, the nucleic acid or amino acid sequence of a homologue, or a mutant, allele or variant (see above) shares homology with the sequence of or encoded by the nucleotide sequence of Figure 4
15 or Figure 6, preferably at least about 50%, or at least about 60%, or at least about 70%, or at least about 75%, or at least about 80% homology, most preferably at least about 90% homology, and the encoded product shares a phenotype with the *cen* and/or
20 *tfl1* gene, preferably the ability to influence switching of apical meristem to a floral fate and/or influence timing of flowering. The influence may promote or delay such switching and/or flowering compared with wild-type. "Homology" may be understood
25 to refer to similarity, in functional terms, in an amino acid sequence, as is standard in the art. Thus, for example, a % similarity figure will include amino acid differences that have little or no functional

significance, such as leucine to isoleucine.

Otherwise, homology may be taken to refer to identity.

For example, gene homologues from economically important monocotyledonous crop plants such as rice
5 and maize may be identified. Although genes encoding the same protein in monocotyledonous and dicotyledonous plants show relatively little homology at the nucleotide level, amino acid sequences are conserved.

10 In certain embodiments, an allele, variant, derivative, mutant or homologue of the specific sequence may show little overall homology, say about 20%, or about 25%, or about 30%, or about 35%, or about 40% or about 45%, with the specific sequence.
15 However, in functionally significant domains or regions the amino acid homology may be much higher. Comparison of the amino acid sequences of the polypeptides reveals domains and regions with functional significance, i.e. a role in influencing a
20 flowering characteristic of a plant, such as switching of apical meristem and/or timing of flowering. Deletion mutagenesis, for example, may be used to test the function of a region of the polypeptide and its role in or necessity for influence of a flowering
25 characteristic such as timing.

Also according to the invention there is provided a plant cell having incorporated into its genome a sequence of nucleotides as provided by the present

invention, under operative control of a promoter for control of expression of the encoded polypeptide. A further aspect of the present invention provides a method of making such a plant cell involving

5 introduction of a vector comprising the sequence of nucleotides into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides into the genome.

10 The present invention further encompasses a plant comprising a plant cell comprising nucleic acid according to the present invention e.g. as a result of introduction of the nucleic acid into the cell or an ancestor thereof, and selfed or hybrid progeny and any
15 descendent of such a plant, also any part or propagule of such a plant, progeny or descendant, including seed.

In certain embodiments, a plant according to the invention may be one which does not breed true.

20 Stability, i.e. the ability to breed true, is one of the requirements of the UPOV Convention for a plant to be subject to Plant Variety Rights. Accordingly, a plant that does not breed true is not a plant variety.

The invention further provides a method of
25 influencing the apical meristem switching and/or other flowering characteristics of a plant comprising expression of a heterologous *cen* or *tfl1* gene sequence (or mutant, allele, derivative or homologue thereof,

as discussed) within cells of the plant. The term "heterologous" indicates that the gene/sequence of nucleotides in question have been introduced into said cells of the plant or an ancestor thereof, using genetic engineering, i.e. by human intervention, for instance using appropriate transformation techniques. The gene may be on an extra-genomic vector or incorporated, preferably stably, into the genome. The heterologous gene may replace an endogenous equivalent gene, ie one which normally performs the same or a similar function or the inserted sequence may be additional to the endogenous gene. An advantage of introduction of a heterologous gene is the ability to place expression of the gene under the control of a promoter of choice, in order to be able to influence gene expression, and therefore plant phenotype, according to preference. Furthermore, mutants, variants and derivatives of the wild-type gene, eg with higher or lower activity than wild-type, may be used in place of the endogenous gene.

The principal characteristics which may be altered using the present invention are controlling the switch of meristems to a floral fate and the timing of flowering. Over-expression of the gene product of the *tf11* gene may lead to delayed flowering; under-expression may lead to precocious flowering. Down-regulation may be achieved, for example, with "gene silencing" techniques such as anti-

sense or sense regulation, discussed further below.

This degree of control is useful to ensure synchronous flowering of male and female parent lines in hybrid production, for example. Another use is to
5 advance or retard the flowering in accordance with the dictates of the climate so as to extend or reduce the growing season. Similarly, switching of apical meristems to a floral fate may be delayed or promoted according to the level of *cen* or *tfl1* gene product.
10 Conversion of indeterminate growth to a terminal flower phenotype on down-regulation of *cen* or *tfl1* may allow for development of a limited number of fruits or seeds which mature, ripen and/or dry in a certain period. This may be beneficial where harvesting of
15 immature, unripe and/or not dry fruit or grains is undesirable. For example, young and unripe canola seeds still containing chlorophyll when the cold falls in and prematurely stops the maturing and ripening process require further and costly refining of the
20 crushed oil which is undesirably green. Grains or fruit crops over-expressing *CEN/Tfl1* may be used for increasing the yield of particular crops. Changing of the architecture, in particular flowers, of ornamental plant species either from determinate to indeterminate
25 or from indeterminate to determinate may be of commercial value.

The nucleic acid according to the invention may be placed under the control of an externally inducible

gene promoter thus placing the timing of meristem switching and/or flowering under the control of the user. The use of an inducible promoter is described below. This is advantageous in that flower
5 production, and subsequent events such as seed set, may be timed to meet market demands, for example, in cut flowers or decorative flowering pot plants. Delaying flowering in pot plants is advantageous to lengthen the period available for transport of the
10 product from the producer to the point of sale and lengthening of the flowering period is an obvious advantage to the purchaser.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In
15 essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus. The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or
20 no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible
25 promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon application of the relevant stimulus by an amount effective to alter a

phenotypic characteristic. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level which brings about the desired phenotype.

Suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level in virtually all plant tissues (Benfey et al, 1990a and 1990b); the maize glutathione-S-transferase isoform II (GST-II-27) gene promoter which is activated in response to application of exogenous safener (WO93/01294, ICI Ltd) (preferred in the present invention); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, eg inner phloem, flower primordia, branching points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana* LEAFY promoter that is expressed very early in flower development (Weigel et al, 1992).

An aspect of the present invention is the use of nucleic acid according to the invention in the production of a transgenic plant.

When introducing a chosen gene construct into a cell, certain considerations must be taken into

account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be
5 available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur. Finally, as far as plants are concerned the target cell type must
10 be such that cells can be regenerated into whole plants.

Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic
15 manipulation of plants. DNA can be transformed into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984),
20 particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green et al. (1987) *Plant Tissue and Cell Culture*, Academic Press),
electroporation (EP 290395, WO 8706614 Gelvin Debeyser
25 - see attached) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman et al. *Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g.

Kindle, *PNAS U.S.A.* 87: 1228 (1990d) Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

- Agrobacterium transformation is widely used by
- 5 those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988)
- 10 *Bio/Technology* 6, 1072-1074; Zhang, et al. (1988) *Plant Cell Rep.* 7, 379-384; Zhang, et al. (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, et al. (1989) *Nature* 338, 274-276; Datta, et al. (1990) *Bio/Technology* 8, 736-740; Christou, et al. (1991)
- 15 *Bio/Technology* 9, 957-962; Peng, et al. (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, et al. (1992) *Plant Cell Rep.* 11, 585-591; Li, et al. (1993) *Plant Cell Rep.* 12, 250-255; Rathore, et al. (1993) *Plant Molecular*
- 20 *Biology* 21, 871-884; Fromm, et al. (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, et al. (1990) *Plant Cell* 2, 603-618; D'Halluin, et al. (1992) *Plant Cell* 4, 1495-1505; Walters, et al. (1992) *Plant Molecular Biology* 18, 189-200; Koziel, et al. (1993)
- 25 *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, et al. (1993) *Plant Physiology* 102, 1077-1084; Somers, et al. (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In

particular, *Agrobacterium* mediated transformation is now emerging also as an highly efficient alternative transformation method in monocots (Hiei et al. (1994) *The Plant Journal* 6, 271-282).

5 The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, et al. (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, 10 *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

 Microprojectile bombardment, electroporation and direct DNA uptake are preferred where *Agrobacterium* is inefficient or ineffective. Alternatively, a 15 combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with *Agrobacterium* coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co- 20 cultivation with *Agrobacterium* (EP-A-486233).

 Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues 25 and organs of the plant. Available techniques are reviewed in Vasil et al., *Cell Culture and Somatic Cell Genetics of Plants*, Vol I, II and III, Laboratory Procedures and Their Applications, Academic Press,

1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will be determined by its efficiency to
5 transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to
10 introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

In the present invention, over-expression may be achieved by introduction of the nucleotide sequence in
15 a sense orientation. Thus, the present invention provides a method of influencing a flowering characteristic, e.g. meristem switching, of a plant, the method comprising causing or allowing expression of the product (polypeptide or nucleic acid) encoded
20 by the nucleotide sequence of nucleic acid according to the invention from that nucleic acid within cells of the plant.

Under-expression of the gene product polypeptide may be achieved using anti-sense technology or "sense
25 regulation". The use of anti-sense genes or partial gene sequences to down-regulate gene expression is now well-established. Double-stranded DNA is placed under the control of a promoter in a "reverse orientation"

such that transcription of the "anti-sense" strand of the DNA yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene. The complementary anti-sense RNA sequence is
5 thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein. Whether or not this is the actual mode of action is still uncertain. However, it is established fact that the technique works. See.
10 for example, Rothstein et al, 1987 *PNAS USA*, 84: 8439-8443; Smith et al, (1988) *Nature* 334, 724-726; Zhang et al, (1992) *The Plant Cell* 4, 1575-1588, English et al., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in reviewed in Bourque,
15 (1995), *Plant Science* 105, 125-149, and Flavell, (1994) *PNAS USA* 91, 3490-3496.

The complete sequence corresponding to the coding sequence in reverse orientation need not be used. For example fragments of sufficient length may be used.
20 It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding sequence to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG
25 codon, and perhaps one or more nucleotides upstream of the initiating codon. A suitable fragment may have about 14-23 nucleotides, e.g. about 15, 16 or 17.

Thus, the present invention also provides a

method of influencing a flowering characteristic, e.g. meristem switching, of a plant, the method comprising causing or allowing anti-sense transcription from nucleic acid according to the invention within cells
5 of the plant.

When additional copies of the target gene are inserted in sense, that is the same, orientation as the target gene, a range of phenotypes is produced which includes individuals where over-expression
10 occurs and some where under-expression of protein from the target gene occurs. When the inserted gene is only part of the endogenous gene the number of under-expressing individuals in the transgenic population increases. The mechanism by which sense
15 regulation occurs, particularly down-regulation, is not well-understood. However, this technique is also well-reported in scientific and patent literature and is used routinely for gene control. See, for example, van der Krol et al., (1990) *The Plant Cell* 2, 291-299;
20 Napoli et al., (1990) *The Plant Cell* 2, 279-289; Zhang et al., (1992) *The Plant Cell* 4, 1575-1588.

Thus, the present invention also provides a method of influencing a flowering and/or meristem switching characteristic of a plant, the method
25 comprising causing or allowing expression (at least transcription) from nucleic acid according to the invention within cells of the plant to suppress activity of a polypeptide with ability to influence a

flowering characteristic. Here the activity of the polypeptide is preferably suppressed as a result of under-expression within the plant cells.

As stated above, the expression pattern of the gene may be altered by fusing it to a foreign promoter. For example, International patent application WO93/01294 of Imperial Chemical Industries Limited describes a chemically inducible gene promoter sequence isolated from a 27 kD subunit of the maize glutathione-S-transferase, isoform II gene (GST-II-27). It has been found that when linked to an exogenous gene and introduced into a plant by transformation, the GST-II-27 promoter provides a means for the external regulation of the expression of that exogenous gene.

The GST-II-27 gene promoter has been shown to be induced by certain chemical compounds which can be applied to growing plants. The promoter is functional in both monocotyledons and dicotyledons. It can therefore be used to control gene expression in a variety of genetically modified plants, including field crops such as canola, sunflower, tobacco, sugarbeet, cotton; cereals such as wheat, barley, rice, maize, sorghum; fruit such as tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, and melons; and vegetables such as carrot, lettuce, cabbage and onion. The GST-II-27 promoter is also suitable for use in a variety of tissues, including

roots, leaves, stems and reproductive tissues.

Accordingly, the present invention provides in a further aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide
5 sequence provided by the present invention. This enables control of expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of
10 expression of a construct within a plant cell, by application of a suitable stimulus, an effective exogenous inducer. The promoter may be the GST-II-27 gene promoter or any other inducible plant promoter.

Ectopic expression of sense constructs may be
15 used to inhibit flowering and convert meristems to indeterminate growth. This is useful for crops whose yield is increased by having a more extensive vegetative phase, especially when expression is later turned off. Limited expression of *cen*, for example
20 under *plena/agamous* promoters, may cause indeterminate stems wrapped in petals, a potentially highly ornate stem.

Anti-sense or co-suppression constructs, mutant selection or other mechanisms to affect gene activity
25 may inhibit *cen* and homologues in different species and convert indeterminate apical meristems to flowers. This may be useful in crops where tops must be pinched-off to promote laterals and "bushy"

development, or where flower number should be limited to give bigger flowers or fruits. The cut flower industry may enjoy new varieties, while the fruit tree and paper tree industries may profit from a change in branching architecture.

As discussed, the *tfl1* gene also has the effect of delaying flowering. Thus, both sense and anti-sense constructs may be used to affect flowering time. In species which benefit from delaying flowering, such as sugar beet and lettuce, or promoting flowering, transgenics may employ *tfl1* or an appropriate homologue or mutant or derivative, as discussed.

The *cen* and *tfl1* genes may be used to modulate the expression of other genes, such as *flo* or *lfy*, whose phenotypes are complementary to *cen/tfl1*, and vice versa.

Both molecular and phenotypic analysis indicate a mutual antagonism between *cen/tfl1* and *flo/lfy*. The normal pattern of flowering depends on how the balance between these two antagonistic activities is established. By manipulating this balance flowering may be controlled in different ways to achieve a desirable result. The phenotype of lines expressing *cen/tfl1* may be modified by changing *flo/lfy* expression and vice versa, either genetically (e.g. by crossing selected phenotypes of plants expressing *cen/tfl1* or homologues thereof with selected phenotypes of plants expressing *flo/lfy* or homologues

thereof) or transgenetically (e.g. by using expression cassettes employing a stronger or weaker promoter to drive *cen/tfl1* as compared to *flo/lfy*). For example, plants overexpressing *cen/tfl1* with a prolonged
5 vegetative phase may be induced to flower by activation of a *flo/lfy* construct under the control of an inducible promoter.

Preliminary analysis reveals that *cen* is restricted in its expression to the apical region
10 lying just below the shoot meristem. The *cen* promoter may therefore be employed in directed expression of genes to the apex, using suitable nucleic acid constructs.

For example, the *cen* promoter may be used to
15 express a suitable phytotoxin to inhibit apical meristem switching into an inflorescence and/or floral meristem thereby preventing bolting and/or flowering.

Suitable phytotoxin for this purpose may include but are not limited to ribosome inhibiting proteins
20 (Lord et al. (1991) Seminars in Cell Biol. 2:15-22, Stirpe et al. (1992) Bio/Technology 10:405-412) such as dianthin (Legname et al. (1991) Biochem. Biophys. Acta 1090:119-122), pokeweed antiviral protein (PAP) (Chen et al. (1993) Physiol. Mol. Plant Pathol.
25 42:237-247), ricin A (Endo and Tsurugi (1988) J.Biol.Chem. 263:8735-8739), ribonucleases such as barnase or RNase T1 (Mariani et al. (1990) Nature 347:737-741, Mariani et al. (1992) Nature 357:384-387)

or a diphtheria toxin A chain (Thorsness et al. (1991) Dev. Biol. 143:173-184).

Accordingly, a further aspect of the present invention provides nucleic acid isolate comprising a
5 *cen* promoter sequence, for instance a promoter sequence shown in Figure 4, or a mutant, derivative, variant, allele or homologue thereof, especially retaining ability to promote tissue-specific expression with a tissue pattern matching or similar
10 to *cen* tissue expression pattern. The predicted promoter lies upstream in Figure 4 of NT 4327, probably within 500 nt of the start codon. The nucleic acid may be a gene construct in which a nucleotide sequence of choice is placed under control
15 of the promoter (using appropriate orientation, spacing and so on) for expression. Techniques for nucleic acid manipulation and plant transformation, and other procedures needed to put into practice this aspect of the present invention, are disclosed above
20 in relation to the *cen* and *tfl1* genes, homologues, mutants and derivatives.

The present invention provides a nucleic acid isolate including or consisting essentially of a promoter, the promoter including the nucleotide
25 sequence shown in Figure 4(b) as nucleotides 1-4417 or a mutant, allele, variant, derivative, homologue, or fragment thereof which confers on the promoter ability to promote apical meristem-specific expression in a

plant.

The promoter may include one or more fragments of the sequence shown in Figure 4(b), sufficient to promote gene expression in the required tissue-specific manner. Restriction enzyme or nucleases may be used to digest the nucleic acid, followed by an appropriate assay (for example involving transforming plants with constructs including a reporter gene such as GUS operably linked to the test sequence) to determine the minimal sequence required. A preferred embodiment of the present invention provides a nucleic acid isolate with the minimal nucleotide sequence shown in Figure 4(b) required for the tissue-specific promoter activity.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter.

The present invention extends to a promoter which has a nucleotide sequence which is allele, mutant, variant or derivative, by way of one or more of nucleotide addition, insertion, substitution and

deletion in a promoter sequence as provided herein. Systematic or random mutagenesis of nucleic acid to make an alteration to the nucleotide sequence may be performed using any technique known to those skilled
5 in the art. One or more alterations to a promoter sequence according to the present invention may increase or decrease promoter activity.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter
10 activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a
15 specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain
20 reaction. Use of a reporter gene facilitates determination of promoter activity by reference to protein production.

In various embodiments of the present invention a promoter which has a sequence that is a fragment,
25 mutant, allele, derivative or variant, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the sequence of the promoter shown in Figure 4(b), has at least about 60% homology

with one or both of the shown sequences, preferably at least about 70% homology, more preferably at least about 80% homology, more preferably at least about 90% homology, more preferably at least about 95% homology.

- 5 The sequence in accordance with an embodiment of the invention may hybridise with one or both of the shown sequences, or the complementary sequences (since DNA is generally double-stranded).

Further provided by the present invention is a
10 nucleic acid construct including or consisting essentially of a promoter according to the invention operably linked to a nucleotide sequence to be expressed, e.g. a coding sequence or sequence encoding desired RNA (e.g. for sense or anti-sense regulation).

- 15 The gene may be heterologous, by which is meant a sequence other than that of *cen.* Generally, the sequence may be transcribed into mRNA which may be translated into a peptide or polypeptide product which may be detected and preferably quantitated following
20 expression. A gene whose encoded product may be assayed following expression is termed a "reporter gene", i.e. a gene which "reports" on promoter activity.

Further provided as aspects of the present
25 invention are vectors constructs and host cells containing nucleic acid including a promoter according to the invention. Host cells may be microbial or plant. Plants comprising such plant cells, whether

varieties or not, are also provided by the present invention, as is the use of the nucleic acid in the production of a transgenic plant. Methods of causing or allowing expression from the promoter in host
5 cells, such as plant cells, which may be in plants, represent further aspects of the invention.

All documents mentioned herein are incorporated by reference.

10

Experimental work which lead to the making of the present invention will now be described with reference to the accompanying figures.

15 Figure 1: Cartoons of *tfl1* mutant and wild-type plants.

In wild-type (Figure 1a), the inflorescence grow indefinitely and flowers (circles) are generated from the periphery of indeterminate inflorescence meristems
20 (filled arrow heads). Secondary inflorescences (coflorescences) arise in the axils of stem leaves. In *tfl1* plants (Figure 1b), inflorescences are often replaced by a single, terminal flower.

25 Figure 2: Genomic DNA blot.

DNA from the wild-type *Antirrhinum* progenitor line (JI.2 WT), the original Gatersleben *cen* allele (*cen*-594) and three new *cen* alleles (663, 665 and 666)

identified in the F1 population arising from a cross between mutagenised JI.WT plants and *cen*-594, were digested with EcoRI, blotted and probed with the flanking region of pJAM2017 (see Figure 3). A wild-type F1 sibling generated in the mutagenesis (sib) and a wild-type revertant (Rev+) arising from the *cen*-594 allele, were treated similarly.

Figure 3: The *cen* locus.

Figure 3 (a) Map of the *cen* genomic region carrying the *cen*-663 allele. The insertions site of the transposon Tam6 is shown with EcoRI, E, and XbaI, X, sites indicated. The internal Tam6 XbaI fragment used to isolate the 6.0 kb EcoRI fragment, segregating with the *cen* phenotype of plants carrying *cen*-663, is flanked by an EcoRI site (E) that only partially cut in genomic DNA digests. This allowed the isolation of the 6.0 kb fragment from *cen*-663 and was cloned as pJAM2017. The 2 kb flanking region (an AccI, A, to EcoRI fragment) used to probe the genomic DNA of Figure 2 is shown as a thicker line below the locus. The 6.5 kb EcoRI wild-type genomic fragment was subcloned as pJAM2018. The 7 kb BamHI, B, was subcloned as pJAM2019. Sequencing of these wild-type clones revealed two regions with similarity to upstream regions of the *Antirrhinum* genes *globosa* and *FIL1*, indicated by open boxes and marked g and f, respectively.

Figure 3 (b) Structure of the *cen* gene and the insertion of the transposon-generated alleles determined by sequencing. Exons are represented by boxes, filled for coding and open for untranslated.

5 Introns are indicated by horizontal lines. Triangles upon vertical lines indicate the transposon insertion sites of the alleles indicated. The arrow shows the direction of transcription.

10 Figure 4

Figure 4(a) shows the nucleotide sequence of *cen* cDNA compiled from 5' and 3' RT-PCR products and comparison with the genomic sequence. The deduced amino acid sequence and the longest open reading frame
15 is shown below.

Figure 4(b) shows the genomic sequence containing the *cen* gene. The *cen* cDNA sequence is given in lower case with the predicted amino acid sequence below. Upper case shows the 5' and 3' regions and the
20 introns. The promoter sequence is included.

Figure 5: Similarly of *cen* to animal lipid-binding proteins.

The amino acid sequences (one letter code) for
25 the deduced protein gene products of *cen* of *Antirrhinum* (*Cen*), morphine- or lipid-binding protein of rats (*Pbpl*) and bovine phosphatidylethanolamine-binding protein (*Pbp*) are shown.

Figure 6:

Figure 6(a) shows the nucleotide sequence of *tfl1* cDNA obtained from an *Arabidopsis* EST, and the predicted encoded amino acid sequence. Point mutations were detected in *tfl1* alleles as indicated, with the underlined base substituted with the base directly above. These mutations result in changes in the encoded amino acid sequence: glycine to aspartate in *tfl1-1*, glycine to serine in *tfl1-11*, glutamate to lysine in *tfl1-13* and threonine to isoleucine in *tfl1-14*.

Figure 6(b) shows the genomic sequence of the *Arabidopsis* clone containing the EST cDNA clone 129D7T7. The EST cDNA sequence is given in lower case with the predicted amino acid sequence below. Upper case shows the 5' and 3' regions and the introns.

Figure 7: *Arabidopsis* and rice Expressed Sequence Tags with similarity to *cen*.

The *Arabidopsis* clone (Arab) was completely sequenced and appeared to be full length, while the rice clone (Rice1946) was only sequenced at the 3' end. Data also suggested that the rice clone was a cDNA from an unprocessed transcript. Therefore, only the likely 3' coding region was translated to give the predicted peptide shown. A separate rice clone from the database, Rice2918, was also likely to be unprocessed and therefore two peptides, a and b,

similar to those of exons 2 and 3 of *cen*, were translated for comparison.

Figure 8: Plasmid constructs for ectopic expression of
5 *cen* and *tfl1*.

The *cen* and *tfl1* open reading frames were cloned downstream of the Cauliflower 35S promoter and inserted into binary vectors (SLJ44024A) to give plasmids pJAM2075 (Figure 8(a)) and pJAM2076 (Figure
10 8(b)) respectively.

Materials and Methods

Plants

The original *cen* allele, *cen*-594, was obtained
15 from Gatersleben, Germany. A derivative of stock JI.2 was used that contained a *globosa* allele. Plants of this JI. line were grown at 15°C and then used in crosses with *cen*-594 also grown at 15°C (Carpenter et al., 1987). Progeny from these crosses were grown and
20 three new *cen* alleles, *cen*-663, *cen*-665 and *cen*-666 were obtained. These F1 plants and three wild-type siblings from each family were maintained as cuttings (Carpenter and Coen, 1995).

25 DNA and RNA Analysis.

The methods for DNA and RNA extraction and blot analysis were as described previously (Coen et al., 1986; Coen and Carpenter, 1988). The Tam6 fragment

used in screening was a 4 kb XbaI fragment which was flanked on either side by EcoRI sites (see map of Figure 3; Doyle et al., unpub). The 6.0 kb EcoRI fragment identified in cen-663 with Tam6 was isolated
5 by digesting genomic DNA from a homozygous cen-663 plant (obtained from selfing of the original F1), fractionating DNA by agarose gel electrophoresis and electroeluting a 5-7 kb size fraction, purifying this by ion-exchange chromatography using a NACS PREPAC
10 column (Bethesda Research Laboratories, Inc.) and ligating to EcoRI digested and phosphatased lambda gt10 arms as described in the Kit protocol (Amersham cDNA rapid cloning module- lambda gt10 code RPN1713). Packaging in vitro (Amersham module N334L) gave a
15 library of about 150,000 recombinants, which was screened using the Tam6 probe. One positive was isolated and purified that contained a 6.0 kb EcoRI fragment, though 3.6 and 2.4 kb bands were present in varying amounts. The 6.0 kb fragment was subcloned in
20 to Bluescript vector KS+ (Stratagene) to give pJAM2017 and, when mapped, revealed an internal EcoRI site that gave 3.6 and 2.4 kb fragments. This suggested that the 6.0 kb band was only partially digested, as expected from the map of Tam6 and the internal XbaI
25 probe used in screening. The region flanking Tam6 (a 2 kb AccI-EcoRI fragment) was used to screen a lambda EMBL4 library of wild-type *Antirrhinum* DNA, partially digested with Sau3A. From about 500,000 recombinants,

7 overlapping clones were isolated, with inserts of average size 15-16 kb. These clones were used to construct a map of the genomic region and to determine the approximate positions of the insertions responsible for the different alleles. Exact insertion sites were determined using PCR on genomic DNA of each allele, with oligonucleotides to *cen* in both directions, and a conserved oligo to the CACTA family of transposable elements (Doyle et al., unpub.). The 6.5 kb genomic clone, pJAM2018, contained the insertion sites of all alleles but did not identify any cDNA clones when used as a probe against a cDNA library constructed from poly(A) RNA isolated from young inflorescences of wild-type *Antirrhinum* (Simon et al., 1994). Therefore, a small region (about 200 bp) flanking the *cen*-663 allele was sequenced by the dideoxynucleotide method (Chen and Seeburg, 1985) using Sequenase version 2 from United States Biochemical Corporation. Oligos based on this sequence were designed in both directions, in possible Open Reading Frames, for RT-PCR on total RNA from wild-type and *cen* mutants young inflorescences. This identified a cDNA originating from the region flanking the insertion in *cen*-663 which was not expressed in each of the alleles. This partial *cen* cDNA was subcloned in to Bluescript vector KS+ as pJAM2020. Both the genomic and cDNA clones were fully sequenced and the intron-exon boundaries determined. The 5' end

of the *cen* mRNA was determined using the kit, 5'RACE system for rapid amplification of cDNA ends (GibcoBRL). The complete *cen* cDNA was constructed from the different RT-PCR products using convenient
5 restriction enzyme sites. Database searches involved BLASTN (Altschul et al., 1990) and FASTA (Pearson and Lipman, 1988).

The *Arabidopsis* clone 129D7T7 was obtained from the *Arabidopsis* Biological Resource Center at Ohio
10 State and was originally isolated from *A.thaliana* var Columbia and partially sequenced by Newman et al., at MSU-DOE, Michigan (Accession No. T44654). The rice clone S1946_1A was obtained from Sasaki et al., National Institution of Agrobiological Resource Rice
15 Genome Resource Project, Ibaraki, Japan and was isolated from *Oryza sativa* (Accession No. D40166). The partial sequence of the rice clone R2918_1A was obtained from the databases. Mapping of the *Arabidopsis* cloned was as described (Schmidt et al.,
20 1994).

In situ Hybridisation

The methods for digoxigenin labelling of RNA probes, tissue preparation and *in situ* hybridisation
25 were as described (Bradley et al., 1993). An internal *AccI*-*RsaI* fragment of the partial *cen* cDNA, pJAM2020, was subcloned in to Bluescript vector KS+ and used to generate antisense and sense control probes using T3

and T7 polymerase. An internal fragment of *tfl* was generated by PCR, subcloned into pGEM-T vector (Promega) to give plasmid pJAM2045, and used to generate antisense and sense probes using T7 and SP6
5 polymerases.

Constructs and transformation

The *cen* and *tfl1* open reading frames were isolated and each used to replace the GUS gene of
10 plasmids SLJ4D4 and SLJ4K1 respectively (Jones et al., 1992). The *cen* and *tfl1* open reading frames, flanked by the CaMV 35S promoter and ocs or nos terminators respectively, were isolated and cloned into the binary vector SLJ44024A (Jones et al., 1992) to give pJAM2075
15 and pJAM2076. Transformation of *Arabidopsis* was made by vacuum infiltration (Bechtold et al., 1993) and root transformation and regeneration (Valvekans et al., 1988).

20 Results

Isolation of new *cen* alleles

Early analysis of the original *cen* allele obtained from Gatersleben (*cen*-594) suggested that it was not very unstable and, therefore, that it might
25 not be transposon-induced. Furthermore, it was in a line that might carry quite a different array of transposons from the probes available to those present in John Innes lines. Therefore, in 1990, a directed-

tagging approach was set up using transposon-active John Innes lines, grown at 15°C, crossed to the Gatersleben allele. The line chosen was a derivative of stock JI.2 and contained a new *globosa* (*glo*) allele, which suggested that transposons were possibly active in the *glo* region. Early mapping data suggested linkage between *cen* and *glo*, so this line may have provided a source of active transposons in the vicinity of *cen* (Stubbe, 1966). Also, because transposons tend to jump to linked sites, the frequency of insertions at *cen* could be enhanced in this line (Coen et al., 1988). In 1992, from a screen of about 10,000 plants, three new alleles of *cen* were successfully isolated in the F1 generation. The production of these alleles provided a unique resource that was instrumental in allowing *cen* to be isolated.

Description of wild type and *cen* mutants

Early development in all *cen* alleles was as wild type; the apical meristem undergoing a vegetative phase producing leaves bearing dormant or further vegetative shoots. Upon flowering the apical meristem in both wild type and *cen* mutants switched to producing modified leaves (bracts) bearing flowers in their axils. However, while wild type maintained this state, the apical meristem of *cen* plants was converted to a flower after a number of axillary flowers had been produced (Figure 1). In greenhouse or controlled

environmental conditions (16hr daylength and 20-25°C) about 5 to 20 axillary flowers were made on the main shoot of each allele, and fewer on lateral shoots. The new alleles showed variation in both the number of
5 axillary flowers made before the terminal flower and in the morphology of the apical flower. A range of symmetries in apical flowers could be found, from radially symmetrical to a morphology closer to that of axillary flowers.

10

Cloning of *cen*

Genomic DNA from *cen*-663 and three wild-type F1 siblings were digested with EcoRI and probed with transposons Tam1 to Tam8. A Tam6 probe gave a 6.0 kb
15 band that was uniquely present in *cen*-663 and linked to the *cen* phenotype. Linkage was established by probing DNA from individuals of an F2 family, from a backcross of *cen*-663 to wild type, stock JI.2. The fragment was cloned by isolating a 5-7kb fraction of
20 EcoRI-digested genomic *cen*-663 DNA, ligating to a lambda vector and screening the resulting library with Tam6. A positive clone was isolated and its insert subcloned in to Bluescript vector KS+ to give pJAM2017. This clone was mapped and the flanking
25 region used as a probe against DNA from different *cen* alleles and wild-type siblings (Figure 2). The expected 6.0 kb band and variable levels of a 2.5 kb band (a derivative of the 6.0 kb fragment, explained

below) were detected in *cen*-663, whereas the wild type progenitor, JI.2, gave a 6.5 kb band. The allele from Gatersleben, *cen*-594, used as the parent in the directed-tagging experiment, gave 8.1 and 2.5 kb bands. As expected, these two bands were present in all F1 *cen* alleles and their wild-type siblings. However, each *cen* mutant had lost the progenitor wild-type band of 6.5 kb. In *cen*-665, a new band of 3.4 kb was present, while *cen*-666 and neither the wild-type or any new band. The *cen*-666 allele was never obtained in a homozygous state and appeared to carry a deletion of unknown size. Proof that we had cloned part of the *cen* locus came from analysis of revertants Progeny of homozygous *cen*-594, *cen*-663 and *cen*-665 grown and selfed at 15°C gave revertant progeny with a wild-type phenotype, indicating that these alleles were each caused by a transposon insertion. The revertants in each case had a restored wild-type band of 6.5 kb and the corresponding mutant band of each allele, as expected from their heterozygous phenotypes (Figure 2).

Overlapping clones from a wild-type genomic library were isolated and used to construct a map of the *cen* region (Figure 3a). The wild-type 6.5 kb EcoRI fragment was subcloned as pJAM2018 and fully sequenced. The insertions causing the different *cen* alleles were first mapped by genomic DNA blots. Using a conserved oligonucleotide (oligo) to the CACTA end

of a family of transposons in *Antirrhinum*, in combination with oligos to the *cen* region (see below), the alleles indicated were precisely mapped (Figure 3b). The different insertions indicated that the
5 right-hand end of the 6.5 kb *EcoRI* fragment was critical to *cen* function. However, when this and other regions of pJAM2018 were used to probe a cDNA library made from poly(A) RNA from wild-type *Antirrhinum* young inflorescences, no hybridising
10 clones were detected. Since RNA blots similarly proved inconclusive, about 200 bp flanking the *cen*-663 allele was sequenced. A number of oligos, based on this sequence and possible open reading frames (ORF) in both directions, were synthesised and used in RT-
15 PCR on total RNA from wild-type *Antirrhinum* or *cen* mutants, young inflorescences or vegetative shoots and leaves. Only oligos pointing in the same direction (left to right, 5' to 3' in the map of Figure 3) gave a PCR product and this was absent from RNA of the *cen*
20 alleles.

The 3' PCR cDNA was cloned as pJAM2020 and the 5' end of the *cen* mRNA was determined by 5' RACE-PCR. The complete predicted cDNA and ORF were determined (Figure 4). The transcription unit consisted of 4
25 exons comprising about 930 bp. The ORF had the potential to encode a 181 amino acid protein of 20.3 kDa Mr. Searches against databases revealed most similarity to a family of lipid-binding proteins

present in animals (Figure 5). Regions of significant similarity extended throughout the protein and a potential nucleotide-binding region was partly conserved (CEN residues 116-132). These proteins may
5 also complex with GTP-binding proteins, but the domains for both functions have not been clearly defined.

Using the *cen* cDNA as a probe, a genomic library of wild-type *Arabidopsis thaliana* var Columbia was
10 probed at moderate stringency. One strongly hybridising clone was isolated and the region most similar to the probe was fully sequenced (Figure 6). Meanwhile, database searches identified an *Arabidopsis* Expressed Sequenced Tags (EST) clone 129D7T7 that had
15 similarity to *cen*. Complete sequencing of the *Arabidopsis* clone revealed the predicted protein (Arab), to be 70% identical and about 82% similar to *cen* (Figure 7). The *Arabidopsis* EST sequence was identical to the genomic clone and was allowed the
20 intron-exon structure to be determined (Figure 6). This was very similar to the *cen* gene, with identical positions for the introns. Further database searches identified two rice clones (S1946_1A and R2918_1A) whose partial sequences appeared to have introns at
25 positions similar to *cen*. These sequences predicted a C-terminal, 60 amino acid peptide with 80% identity to the end of *cen* for Rice1946, and two predicted peptides (Rice2918a and b) that showed high similarity

to exons 2 and 3 of *cen* (Figure 7).

Identifying *tfl1* as a homologue to *cen*

The Arabidopsis clone, 129D7T7, was mapped using
5 the closest available RFLP and YAC markers to the end
of the chromosome 5. The *tfl1* mutation maps to this
region. Primers based on this sequence were used in
PCR to isolate the corresponding genomic region in
four alleles of *tfl1* (*tfl1*-1, *tfl1*-11, *tfl1*-13
10 *tfl1*-14).

For sequence comparison of the different *tfl1*
alleles, wild-type Arabidopsis (Columbia) and plants
carrying *tfl1* alleles -1, -11, -13 or -14, were grown
on soil under long days, and genomic DNA was isolated
15 using a miniprep method. Leaf tissue was homogenised
while frozen, buffer added (50 mM EDTA, 0.1M Tris-HCL
pH8, 1% SDS) and the sample thawed at 65°C for 2 min.
DNA was extracted with phenol, phenol-chloroform,
chloroform, and precipitated with isopropanol/Na
20 acetate. After an ethanol wash, DNA was resuspended
in TE containing RNase. Oligonucleotide primers were
designed to sequences about 160 bp upstream of the ATG
and 120 bp downstream of the stop codon. To avoid PCR
artefacts, three separate PCRs were carried out on
25 each DNA preparation and one PCR product from each was
cloned into pGEM-T vector (Promega). Each clone of
about 1.3 kb was sequenced using the ABI Prism system
(Perkin-Elmer) and only base changes present in all 3

PCR products for any one allele were considered genuine.

All four alleles show mutations that would disrupt the predicted *Arabidopsis* protein, proving
5 that this gene is *tfl1*. The changes are shown in Figure 6(a), and were single nucleotide mutations as indicated in the figure, resulting in the following amino acid changes: in *tfl1-1* - glycine to aspartate, in *tfl1-11* - glycine to serine, in *tfl1-13* - glutamate
10 to lysine, and in *tfl1-14* - threonine to isoleucine. (The mutant sequences, both nucleotide and amino acid, each represent an aspect of the present invention.)

Expression studies of *cen* and *tfl1*

15 The timing and histological distribution of *cen* and *tfl1* RNA was determined by *in situ* hybridisation using digoxigenin-labelled *cen* on *tfl1* antisense RNA against wild-type tissue of *Antirrhinum* and *Arabidopsis* respectively. In wild-type, *cen* and
20 *tfl1* are expressed in the shoot apex of young inflorescences, in the region immediately below the apical meristem.

Ectopic expression of *tfl1* and *cen* in *Arabidopsis*

25 To overexpress *cen* and *tfl1*, their respective open reading frames were cloned downstream of the Cauliflower 35 S promoter and inserted into binary vectors to give plasmids pJAM2075 and pJAM2076 (Figure

8) and used for transformation. One transformant was obtained with the 35S-cen construct and showed a delay in bolting and flowering and a conversion of flowers to leafy shoots. Six transformants were obtained with 35S-tfl and all showed a conversion of flowers to leafy shoots. They also displayed a range of flowering and bolting times and in the most severe cases, flowering was greatly delayed compared to wild type (more than double the normal number of rosette leaves). Taken together these results show that ectopic expression of *cen* or *tfl1* can delay flowering. Furthermore, the ability of the *cen* gene of *Antirrhinum* to modify flowering time in *Arabidopsis* shows that these genes can act across wide taxonomic distances.

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CLAIMS:

1. A nucleic acid isolate having a nucleotide sequence coding for a polypeptide which includes the amino acid sequence shown in Figure 4(a).
- 5 2. Nucleic acid according to claim 1 wherein the coding sequence is the coding sequence shown in Figure 4(a).
- 10 3. Nucleic acid according to claim 1 wherein the coding sequence is a mutant, allele or variant of the coding sequence shown in Figure 4(a).
- 15 4. A nucleic acid isolate having a nucleotide sequence coding for a product which includes a sequence mutant, allele, variant or derivative of the CEN amino acid or nucleotide sequence of the species *Antirrhinum majus* shown in Figure 4(a) or a homologue from another species, by way of insertion, deletion, addition or substitution of one or more residues, or a
20 said homologue, wherein expression of said product in a transgenic plant influences flowering characteristics of said plant.
- 25 5. Nucleic acid according to claim 4 wherein said flowering characteristics include the switching of apical meristem to a floral fate.

6. Nucleic acid according to claim 5 wherein said product has the ability to inhibit apical meristem from switching to a floral fate.
- 5 7. Nucleic acid according to claim 5 wherein said product has the ability to promote apical meristem switching to a floral fate.
8. Nucleic acid according to claim 4 or claim 5
10 wherein said flowering characteristics include the timing of flowering.
9. Nucleic acid according to claim 8 wherein said product has the ability to advance flowering in a
15 plant.
10. Nucleic acid according to claim 8 wherein said product has the ability to delay flowering in a plant.
- 20 11. Nucleic acid according to claim 5 wherein said homologue is an *Arabidopsis* homologue.
12. Nucleic acid according to claim 11 wherein said homologue encodes the amino acid sequence shown in
25 Figure 6(a).
13. Nucleic acid according to claim 12 having the coding sequence shown in Figure 6(a).

14. Nucleic acid according to claim 13 wherein the coding sequence is a mutant, allele, variant or derivative of the coding sequence of Figure 6(a).

5 15. A nucleic acid isolate comprising a nucleotide sequence coding for a product comprising a sequence mutant, allele, variant or derivative of the product encoded by the nucleic acid of claim 13, by way of insertion, deletion, addition or substitution of one
10 or more residues, which mutant, allele, variant or derivative has at least about 70% homology with the amino acid sequence or nucleotide sequence of Figure 6(a) and ability to influence a flowering characteristic of a plant.

15

16. Nucleic acid according to any of claims 1 to 15 further including a regulatory sequence for expression of said coding sequence.

20 17. Nucleic acid according to claim 16 wherein the regulatory sequence includes an inducible promoter.

18. A nucleic acid isolate having a nucleotide sequence complementary to a coding sequence of any of
25 claims 1 to 15, or a fragment of a said coding sequence suitable for use in anti-sense regulation of expression.

19. Nucleic acid according to claim 18 wherein said nucleotide sequence complementary to a said coding sequence or a fragment thereof is under control of a regulatory sequence for anti-sense transcription.

5

20. Nucleic acid according to claim 19 wherein the regulatory sequence includes an inducible promoter.

21. A nucleic acid vector suitable for transformation
10 of a plant cell and including nucleic acid according to any preceding claim.

22. A host cell containing heterologous nucleic acid according to any preceding claim.

15

23. A host cell according to claim 22 which is microbial.

24. A host cell according to claim 23 which is a
20 plant cell.

25. A plant cell according to claim 24 having said heterologous nucleic acid within its genome.

25 26. A plant cell according to claim 25 having more than one said nucleotide sequence per haploid genome.

27. A plant comprising a plant cell according to any

of claims 24 to 26.

28. A plant according to claim 27 which does not breed true.

5

29. Selfed or hybrid progeny or a descendant of a plant according to claim 27 or claim 28, or any part or propagule of such a plant, progeny or descendant, such as seed.

10

30. A method of influencing a flowering characteristic of a plant, the method comprising causing or allowing expression of a product encoded by heterologous nucleic acid according to any of claims 1 to 17 within cells of the plant.

31. A method of influencing a flowering characteristic of a plant, the method comprising causing or allowing transcription from heterologous nucleic acid according to any of claims 1 to 17 within cells of the plant.

32. A method of influencing a flowering characteristic of a plant, the method comprising causing or allowing anti-sense transcription from nucleic acid according to any of claims 18 to 20 within cells of the plant.

33. Use of nucleic acid according to any of claims 1 to 17 in the production of a transgenic plant.
34. Use of nucleic acid according to any of claims 18 to 20 in the production of a transgenic plant.
35. A nucleic acid isolate including a promoter, the promoter including the nucleotide sequence shown in Figure 4(b) as nucleotides 1-4417 or a mutant, allele, variant, derivative, homologue, or fragment thereof which confers on the promoter ability to promote apical meristem-specific expression in a plant.

Fig.1 (a).

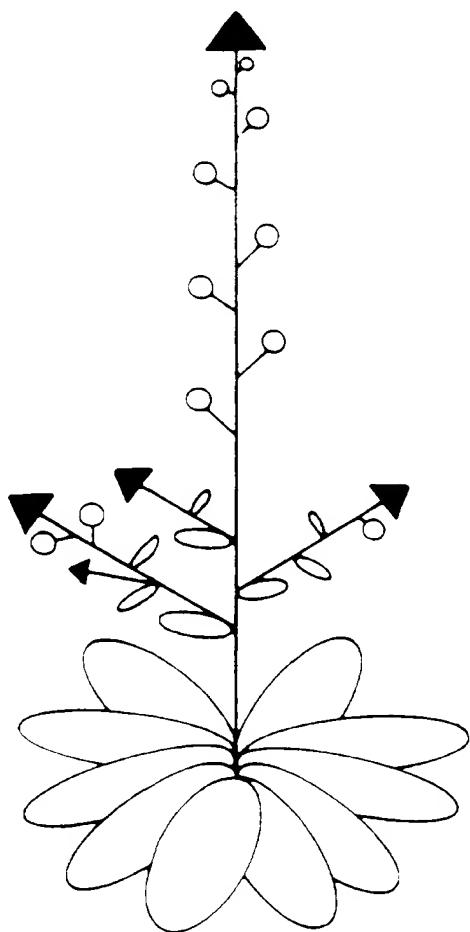
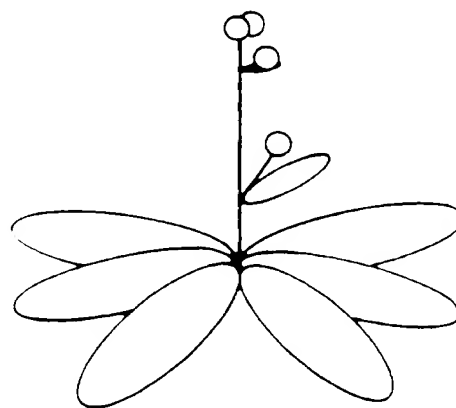
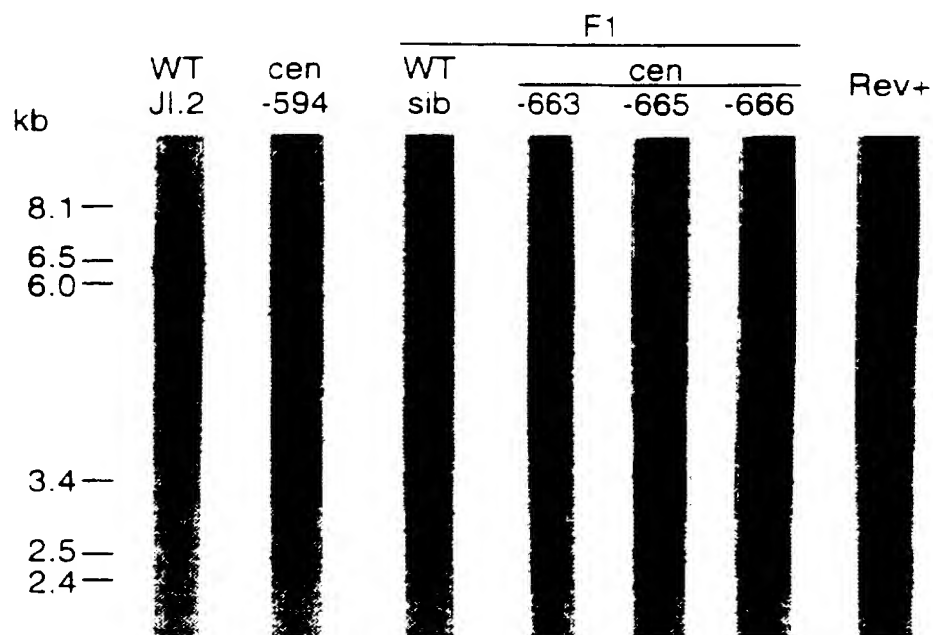


Fig.1 (b).



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Fig.2.



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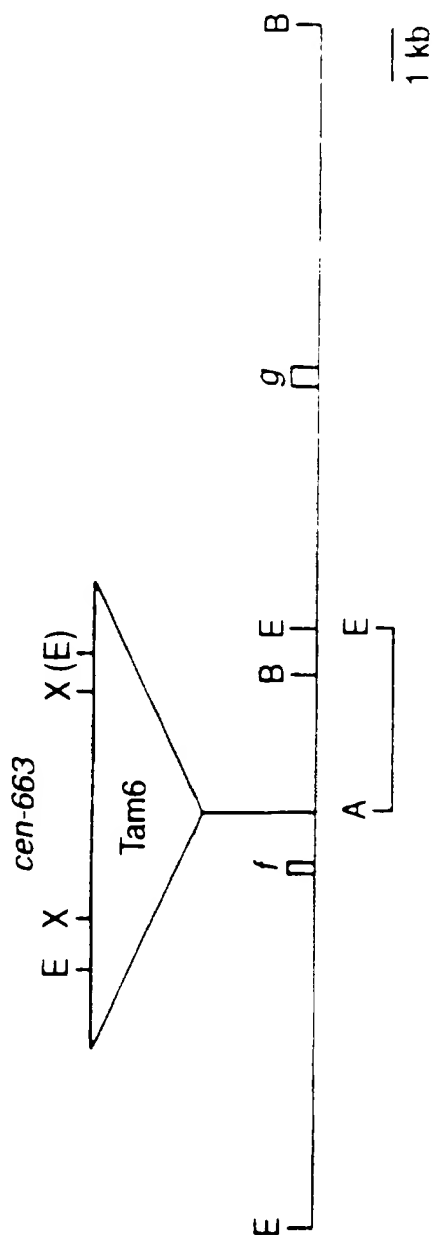


Fig.3 (a).

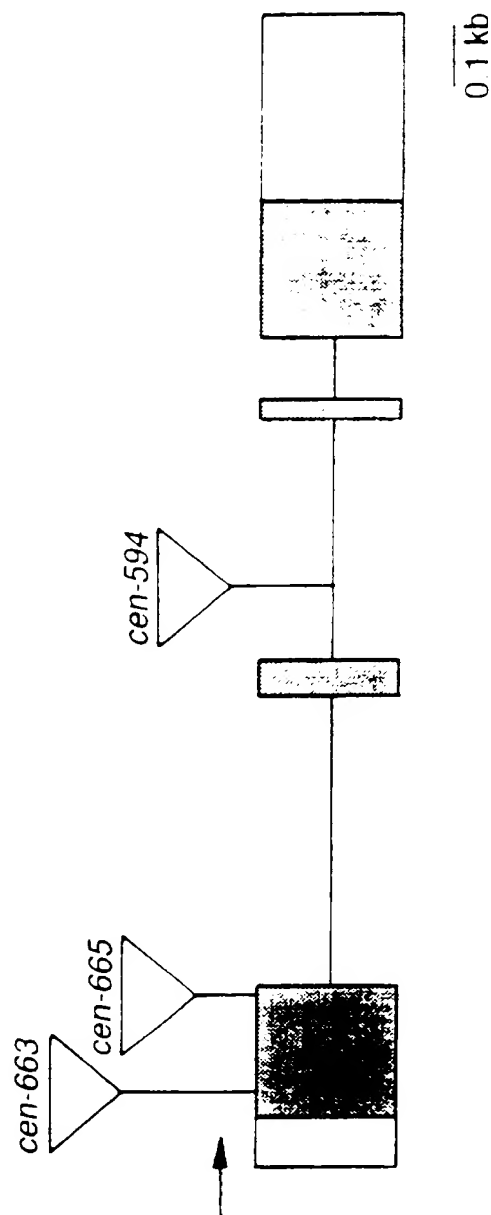


Fig.3 (b).

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Figure 4(a)

1 aagcaacatcaaaaacagcatcataaatcccttttacttttggttgcatTTTTatcatctta 60
61 attaagcattcttctccatataatatagttatggcagcaaaagtttcatcggaccgccta 120
21 M A A K V S S D P L 40
121 gtgatagggagagttatcggagacgttqttgatcattttacctcaactgttaaaatgtct 180
41 V I G R V I G D V V D H F T S T V K M S 60
181 gttatttacaactccaacaattccatcaagcatgtctacaatggccatgagctcttttctt 240
61 V I Y N S N N S I K H V Y N G H E L F P 80
241 tccgctgttacctctacacctaggggttgagggttcaggtggtgatatgagatcatttttc 300
81 S A V T S T P R V E V H G G D M R S F F 100
301 actctgataatgacagaccctgatgttccctggctcctagtgatccataacctgagggagcac 360
101 T L I M T D P D V P G P S D P Y L R E R 120
361 ttgcactggatagtcacagatatcccagggaaccactgattccctcattcggcaagaagta 420
121 L H W I V T D I P G T T D S S F G K E V 140
421 gtgagctatgagatgccaaaggccgaacatagggatccacaggtttgtatttcttctgttc 480
141 V S Y E M P R P N I G I H R F V F L L F 160
481 aaacagaagaaaagagggcaggcgatgttgagccaccagtagtgtgcagggatggattc 540
161 K Q K K R G Q A M L S P P V V C R D G F 180
541 aacacgagaaaattcacacaggaaaatgaattgggcctccctgttgccgctgtcttcttc 600
181 N T R K F T Q E N E L G L P V A A V F F 200
601 aattgccagcgcgaaaccgctgccagaaggcggttgaaacgtactatTTTatccatatcttat 660
201 N C Q R E T A A R R R * 220
661 ggctctgcataatatatatatatatgctagtactactgatgtatcttcatcagggaaat 720
721 aaatcatatgttagggtttcttttgcaatgataaagagtcacctacgtctgctaccaaaaaa 780
781 aattgttagagtggcctttgcaagtagtgaaaggatatgtgtacgtaatagggaaggaaa 840
841 agatggagaaatgggaaattgtgatgtccacttgttataaattgatgtaattaatttcta 900
901 tgatatataatttggaagttgtgtgtgc 929

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Figure 4(b)

1 TCATGTAACATGAAATCACTACCCCTTACATGTGTCTTGGGCAGACGAAGTGGCCTCAATT 60
61 CTAATTGAGCAAACATGGATAGGCAAACGAAACAAGACTTAGAAGACATTAAATCAATTT 120
121 GCTCTAAGTAATGATCGAATTTGAGGTTAAAGGAGTGAATTACACTTCTTTAGCCAAATA 180
181 TCAAATTGTACTTTTTAATACTCAATTTTTATTCAATTTGAAGACTGTGACTTCTTTAGT 240
241 TCTTTTATTGTCTTCTTCTCTTGTGATATATTACTTTTTATTGAAAACGCTCGTTGAATA 300
301 ATATGCAAGCATATGATTAATTCAACCCCTCATATATCCGGTTGATTCTAATATTTTG 360
361 CAAGAAGGACCACAACCCTTAGTTGGTTTTTCGTTTTCTTTTGTCTGACTTCCACTG 420
421 CCCTTGTTTTCAAATTTAATCAGACAAGAATTGGGACAGATAATTTGAATATTTCAATT 480
481 CAGGGAAAAAGGAAATAAGAAATTACAGCTCGTTCTTTTAGAATGAATTAAAGTATTA 540
541 AACAATTGGTACTTTGTGAAAACTACCACATCGTTACCGCTCTTATACCATTAAACC 600
601 CAAACCATTAAATTGATTTTGGAACTTTTCAAATTAATGATGTTTTAATTGCAACAAGTA 660
661 ATTTGCTAGCATTTTAATCTATTTTATCTTCTATGTTACTTGTAGCAACACAACACCTTT 720
721 TCGTGTGCTGTTATCAGATTTTGTATTCTCAATTATCGTATAAACCGTGAAGATATGCCC 780
781 CTCGATCCACGGTCTTAAGCTTTCAATTATTTGAATATTGGAATCTTTGTCTCGGGTTTA 840
841 TACCTGCAGCCAAGATATTCTCAATGTGCCATTCTTGGGTGCCATTTCATCCCTAATTAG 900
901 AAATTACGAATTTTTTTTTTAAAATTTCTAGCACGGAAAGTTGTCTGTTTGAAAAGACC 960
961 AACTCGTGTATTTTATGCTATTGGCCAATTAGTTAATTTGTCATTTCTTTTTTTTTTTTG 1020
1021 TAATGTAAATTTTAGAATATGAAAGCACTAATGATTATGATGAAGTAAACACTTGTTAAT 1080
1081 TTTGATTCCTTTTCTTTTCTTTTAATATTTTCAGATATGTTTATAATTATTCATTAACAT 1140
1141 TTAATTATTCTTTTACTTTCTTTCCCACTTAAACATGAATTAAGAATGTTATTATGTTAT 1200
1201 TATGTAAAAAATTACAAACGTGCGCATTTTTATTTCTCTCTCTAAGCTCATGAATATATA 1260

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Figure 4(b) Continued

1261	ATAATAATTTATTAACATTTAAACAAATATATATCTGTAGAGATAAAGAAAAAAAGTATT	1320
1321	ACCATCACACATATCATAGGAATATGCACCAGGATGGTGAGAAATAATAAGGTTGAAGTA	1380
1381	AAGAAAGATGACGAAAATGAAAAGAAAAAAGAAAAAATTAAAAAGGAGAAATTAT	1440
1441	ATGAGTTAGTTTGTTAATGCACCACTTATATAACCTTTAAAATAAATCATACCCCTTTTA	1500
1501	AAAGTGAATGTACAACACCCTTATGAATTGGATGAGGAGTTGTTCAAGTATGGGGCATT	1560
1561	TATTTATAATATAATATAAAGGAGTTTCAATTGAATAATATCTAATGAAAAATATTGTTG	1620
1621	GGTGTAATTTCTTGAACGATGATGGTGTATCTCATACTTTTTACAAATATGTATGGTC	1680
1681	ACAGTTTATAATTATATATCTAAACATGTATATGTAACTGAATATTGGCAAAGTATAT	1740
1741	TGTACGGCCCAGGTATAAACTTATTATAGGGAAGATAAGCATTGTCTACTATATCACC	1800
1801	CCTTATTCGGTTAAGGCCCACTTGATACTCCATTGGGCCTGAAGAGATTTCTTGAAAAG	1860
1861	CCTACTAACATTTGGGGCTTGAGGACGAGGTTGAGTCCTGAATGGAGAATTTACATGAA	1920
1921	CCAGGATATGTAAGCGGTCCAAAAGGCCCAAATTAATATAATTGATTTTATTATTACTA	1980
1981	AGTTCTATGCAGTAGTTGATTTGTTATCATTTGTTTATCCACGTTATTAAGGATTACCTGA	2040
2041	GTTTATTTGTTTCTACTTCTCATTCTAATCCTGAATTTTAGAAAAATGATCCTACCTC	2100
2101	ACATATGTTAAGACTAAAATTTAATTTCTAGCAAAGTTTCGATTTATTGGAACCAGAAA	2160
2161	GCTCTTTATGTCAATCAGCAATGAGCATAACTTTCTTCTCCATCCAATGATTCATAATTA	2220
2221	GATGATTAACAAATGATTAAGTGCAATATGAGTCACGAATCATCGAGTATTGTTCCCTATT	2280
2281	ATTTAGTTATCAAATTAATCTAAGCATTTCCTCCGTCGAAGTTCAAATATGTCATATTAT	2340
2341	AAACGGAATTATGCCACCATACAATCTTAATATGTACGACGATTCTTTCGAGTTGCGACA	2400
2401	AATAGTTCTTAGCACTGACTTAAATTAAGGACCCTCTGAAGATATAGCAGAATATTACCG	2460
2461	TGTGTATATATATTATTCAATGACCAAAGTGAAGCTCATTAATAATATAGAATTTAATTA	2520

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Figure 4(b) Continued

2521	CCGTGTAACCACATTC	2580
2581	ATATTACGTATAACTTGTAATCAAAGGTTGGCTTAATAGTGTAAGATCCTATTGAGTTC	2640
2641	TCACGGSTGGATGCGATCTATTTAGCAAAACGTCACGAATTTGATCCCTAGCATGTGCAA	2700
2701	ATTTCAATTGCGTCAGTACAACCATGATTGCTGAGCAAAAAATTGTTATTTTCGGGGTGCA	2760
2761	CTTTAAAAAATTCGGGCAGAGTGTGAGACATAAATTGAACTTTTTGTCTTTAAAAACGATA	2820
2821	TTGCCCCGTTACGGTGCTAACCTAATACTATATTTTAAGTAATCGTTTCATAAGTATACA	2880
2881	CGTATAAGTAAAAATAATAGCAAAATGAGCGTATTGAGCTCACCGTTTTTGAATAAAATA	2940
2941	ACAAATTTACATCGGATGAGAACCGCATCGCCGCAGGAAAAAAGAAGGGTGAAGGAGAGA	3000
3001	GATACAAATAAGAAGAAGCAAAAGCTTGAGTATAGATACTCAAGGTATAGAAGTCAAGTT	3060
3061	CAACTAGAGCAAACCTATTAAGAAATTAAATAAAGCATTAGGACTTACTTCTTATAGCAAA	3120
3121	CGAACCCCTCCCCCACCTTGCTACATTAGGGATAGCTAAAACTCAAAATTTATTCCCTTCT	3180
3181	TTTCGTTGAGATGACCTCTCAACTCATTGTAAATGACATGCCATCAATTGTGGAGTTCC	3240
3241	TTTTATGTATGCGCTGATGAAACCTTCTTTATTTATTCTCCTCATATACACACAAATGTC	3300
3301	ATGCTGGAGAACCCTTAGAACCTCCACTTTTATTCTTAAATACAAAAGCTCATAACTCTT	3360
3361	TTGGTAGCTGCAAATGTGCAAACAGTATCCAGAAATTCTATTTGCCCTTTCTTTACATTA	3420
3421	AAAAAGGAATTACAAAGATGAACATCCTCACCTATAGAAATTAATGGGGTAATAGCAAA	3480
3481	AAGTACTCGATGTTATTTCTAATTGGCAAAAGAATCACTGTGTTATTTTAATTAGCAAAA	3540
3541	GAACCTTGTCTTATTTCGGTAAATGGCAAGAAAAAAATTGGCTTCTAGTTTGGAACCTACAC	3600
3601	ATGGTCAATGTGAGTCTTTGCTCCTGACTTACAACCATTTTGTATGATTTTCCCCACTCT	3660
3661	TCCGTAATGCTTCAGTGTTTTAATAAAATTAGCAAAAAACATCCCCTTGTGTTTTTATGA	3720
3721	AATTGGCAATAACCTCCCTGTGTTTCATATAATTGGCAATAACCCCTCTTCTATATACG	3780

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Figure 4(b) Continued

3781	TTTCCTTCAATCAGATGTATCAATTTTCACGGGGTTCGAGGAAGTAAGCTTAAAAAGCATA	3840
3841	ATTTTACCTGCTATTAACGCCCAAAAACAAAATGAGAATATGCTAATTATCGAAAAACAC	3900
3901	ATGCATGTTCTTTTTTTTGCCAATCAAAATGACATTGGGGGTTTATTGTCAATTAAAAATA	3960
3961	ACACGAGGCTAGTTTTTGTTAATAGCTCAGAAATCAATACCTAATTAACCACGCAGTATT	4020
4021	AATTTTACATTTTATGTGAGTGTGAGAGATATAAGAGATACATAAGCGTGGCATGTCA	4080
4081	AAATCATCTTTAATAAGTATACTTCTTGCTTTTGTATATTTTTTTTTTCCAAAAGAAAAA	4140
4141	ACATTCGTCGTAGCTTGGTTGCCTGCCAGATAATGTCTAAAACCAATGTGTCATAGCTAG	4200
4201	ATGGCTGGGTTTTACCCACTTTGAAACTCCCTTAATTCAGTATTTTAATCAAAATTCTCC	4260
4261	TCGCACTGCAATGATCTGCGAGTTGCTTGTAGCCACTATAAATATATGGGGTTTGCTATT	4320
4321	CCATTCTAagcaacatcaaaaaacagcatcataaatccttttacttttgttgcatTTTTAT	4380
4381	catcttaattaagcattctctccatataaatatagttatggcagcaaaagtttcatcgga	4440
1461	M A A K V S S D	1480
4441	cccgtagtgatagggagagttatcgggagacgttggtgatcattttacctcaactgttaa	4500
1481	P L V I G R V I G D V V D H F T S T V K	1500
4501	aatgtctgttattttacaactccaacaattccatcaagcatgtctacaatggccatgagct	4560
1501	M S V I Y N S N N S I K H V Y N G H E L	1520
4561	ctttccttcgctgttacctctacacctagggttgaggttcattggtggtgatagagatc	4620
1521	F P S A V T S T P R V E V H G G D M R S	1540
4621	atttttcactctgGTATTGTTTTACTATTCTGTGCTACTTATCTCTTAGGTTAATTATTG	4680
1541	F F T L	1560
4681	TGAACTCTCTATACCCTAAAATGAAAGATATTTTTGAACCTTCAATGTAATAAGTTCTAC	4740
4741	ATGTGAGGTTCTATCAAAATTTATCTATCAAAATTGTGCAATACTTTTTGTAGTGTTAC	4800
4801	TAGATATATGTCATGTGTAAATATGATAAATACAAGATAAAAACTTAGATACTTTTTTCT	4860
4861	CTATCCACCCATCACTGCATGCATGGATTAAGGTCACGCCATACATTATATACACATGTC	4920
4921	GTTACTCTAATAGCGATATATAGAGTGGTAACGATTTTTTGGTACAGAAATGGTGCTGTA	4980

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Figure 4(b) Continued

4981	AGTTATACAGATGTTTACACAACCACTTAAACTTTTCGTAGTTTTGAGGAATGTTATTTAGT	5040
5041	GTGTAGAATATTTAATATCTTGAAGCAATTAATTTTGAGAGATTTACTCAATTAGTTTGT	5100
5101	TTGTTTCAGataatgacagaccctgatgttccctggtccctagtgateacatacctgagggag	5160
1701	I M T D P D V P G P S D F Y L R E	1720
5161	cacttgcaactgGTAAATATGCTTACTTTGGAACCTTCTTCACACACTAGAAAAATAACAC	5220
1721	H L H W	1740
5221	AAAAGATCATCAAGCCCTAAATTTTTCTTGCATGGAGGAACATATATAACAGGGATTCT	5280
5281	TTCACATTGAGTAAACAAAAGTCACTAGCGAAATGTATAGCTAACCAGTTTATGACAATT	5340
5341	CAAGCTGTTTTAATCATTCTTCCAATTAATGGCCATATATATATATATATATACTCCC	5400
5401	GATAAAAAATGAATCTTTTCAAGAAAATTTTGTGAGCTGCAATGATTCAATCAGCTTTCT	5460
5461	TGAAAATCCCATAAAAGAAATGAACAACTTGCTAATTATGCATTTGATACTTAAAGAGTA	5520
5521	CAAGTTTAATTATGTCACCCCGCTGATATAACTTGATTTGACTAACTCGCAGgatatgtca	5580
1841	I V T	1860
5581	cagatatcccgaggaccactgatctcctcattcgGTATGATTAAATTTTCCCTCCACATTT	5640
1861	D I P G T T D S S F G	1880
5641	AAACCAAATACATTAATAATAATACCCAAATAAATATTCCACCATGACTAATTAATTAA	5700
5701	TAAATTGTTGCAGgcaaagaagtagtgagctatgagatgccaaaggccgaacatagggatc	5760
1901	K E V V S Y E M P R P N I G I	1920
5761	cacagggtttgtatttcttctgttcaaacagaagaaaagagggcaggcgatgttgagccca	5820
1921	H R F V F L L F K Q K K R G Q A M L S P	1940
5821	ccagtagtggtgcagggatggattcaacacgagaaaattcacacaggaaaatgaattgggc	5880
1941	P V V C R D G F N T R K F T Q E N E L G	1960
5881	ctccctgttgccgctgtcttcttcaattgccagcgcgaaaccgctgccagaaggcggttga	5940
1961	L P V A A V F F N C Q R E T A A R R R *	1980
5941	acgtactatccatccatcttcttggtctctgcataatatatatatatatgctagtacta	6000
6001	ctgatgtatcttcatcagggaataaatacatatgtagggttctcttttgcaatgataaaga	6060
6061	gtccctacgtctgctacccaaaaaaattggttagagtggcctttgcaagtagtgaaaggat	6120

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Figure 4(b) Continued

6121 atgtgtacgtaataggggaaggaaaagatggagaaatgggaaattgtgatgtccacttgtt 6180
6181 ataaattgatgtaattaatttctatgatataataatttggagttgtgttgtgcAAAATTTT 6240
6241 GAAGGGCTTAATTTTTGAATGGTTGCAAAAATTATTCCTTTATCTTTTCTTTTAAAACGT 6300
6301 GGAAGCACAATCATTAAATGTCTCTTTGTTTGGTAAACATTTATGTGTATGTCTACAATTT 6360
6361 TTATCGTTTATTTGTACTAATAATTTTAGTTTCGAACATGCAATGTTTGACCTTTTCCTA 6420
6421 TCCGATTGATCATGTGGTTTTTTGATATTATTCTTTGAAGAGTGCTTATGCTTGTTCAGGG 6480
6481 CGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGG 6527

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Figure 5

Gen	HA A K V S	S D	PL V I G R V I G D	V V D H F T S T V K	D S V I Y N S N N S	I K H V Y N G R E L	F P S A V T S T P	57
Pp1	HA A D I S W A G		PL S L Q E V . D E	P P Q H A	L R V D Y G G V T V	DEL G K V L	T P T Q V M N R P S	51
Pbp	P V D L S K W S G		PL S L Q E V . D E	R P Q H P	L Q V K Y G G A E V	DEL G K V L	T P T Q V K N R R P T	50
Gen	R V E V H R G G D M R	S D	PL V I G R V I G D	D V P S P S D P Y L	RE H L H M V V T D	M P G T T D S S	S G K E V V S Y E	114
Pp1	S I S W D G L D P G		K L Y T L V L T D P	D A P S R K D P K F	RE V H H F L V V N	H K G N D I S S G T	V L S E Y V G S	109
Pbp	S I T W D G L D P G		K L Y T L V L T D P	D A P S R K D P K Y	RE V H H F L V V N	H K G N I S S G T	V L S D Y V G S	108
Gen	M P R P N I G E H R	S D	PL V I G R V I G D	K R E I L S	P P V V C R L G F	N T R K F T Q E N E	L G L P V A N V E F	170
Pp1	G P P K D T G L H R		Y V N L V Y E Q E Q	P L M C D L F I L S	H K S G D M R G K F	K V E S F R K K Y H	L G A P V A G T C F	169
Pbp	G P P K G T G L H R		Y V N L V Y E Q E G	P L K C D E P I L S	M R S G D M R G K E	K V A S F R K K Y E	L G A P V A G T C M	168
Gen	N C Q R E T A A R R	R 181						
Pp1	Q A E W D D S V P K	L H D Q L A G K 187						
Pbp	Q A E W D D Y V P K	L Y E Q L S G K 186						

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Figure 6(a)

```

agttaacaaaagaaa atg gag aat atg gga act aga gtg ata gag 45
Met Glu Asn Met Gly Thr Arg Val Ile Glu 10

cca ttg ata atg ggg aga gtg gta gga gat gtt ctt gat ttc ttc 90
Pro Leu Ile Met Gly Arg Val Val Gly Asp Val Leu Asp Phe Phe 25

act cca aca act aag atg aat gtt agt tat aac aag aag caa gtc 135
Thr Pro Thr Thr Lys Met Asn Val Ser Tyr Asn Lys Lys Gln Val 40

tcc aat ggc cat gag ctc ttt cct tct tct gtt tcc tcc aag cct 180
Ser Asn Gly His Glu Leu Phe Pro Ser Ser Val Ser Ser Lys Pro 55

agg gtt gag atc cat ggt ggt gat ctc aga tcc ttc ttc act ttg 225
Arg Val Glu Ile His Gly Gly Asp Leu Arg Ser Phe Phe Thr Leu 70

gtg atg ata gac cca gat gtt cca ggt cct agt gac ccc ttt cta 270
Val Met Ile Asp Pro Asp Val Pro Gly Pro Ser Asp Pro Phe Leu 85

a (tfll-13)
aaa gaa cac ctg cac tgg atc gtt aca aac att ccc ggc aca aca 315
Lys Glu His Leu His Trp Ile Val Thr Asn Ile Pro Gly Thr Thr 100

a (tfll-1)
gat gct acg ttt ggc aaa gag gtg gtg agc tat gaa ttg cca agg 360
Asp Ala Thr Phe Gly Lys Glu Val Val Ser Tyr Glu Leu Pro Arg 115

cca agc ata ggg ata cat agg ttt gtg ttt gtt ctg ttc agg cag 405
Pro Ser Ile Gly Ile His Arg Phe Val Phe Val Leu Phe Arg Gln 130

aag caa aga cgt gtt atc ttt cct aat atc cct tcg aga gat cac 450
Lys Gln Arg Arg Val Ile Phe Pro Asn Ile Pro Ser Arg Asp His 145

ttc aac act cgt aaa ttt gcg gtc gag tat gat ctt ggt ctc cct 495
Phe Asn Thr Arg Lys Phe Ala Val Glu Tyr Asp Leu Gly Leu Pro 160

gtc gcg gcc gtc ttc ttt aac gca caa aga gaa acc gct gca cgc 540
Val Ala Ala Val Phe Phe Asn Ala Gln Arg Glu Thr Ala Ala Arg 175

aaa cgc tag ttt cat gat tgt cat aaa ctg caa aaa tga aag aag 585
Lys Arg * 177

aaaatttgcatgtaatctcatgtttatttgtgttctgaatttccgtactctgaataaaa 644
actgccaaagatgagttgaatccg 668

```


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Figure 6(b)

1 cttagtattagaaataatgtagttagtgtagtgcgtacgtacgtgttgagacagcaaaaaaataa 60
61 aagaaagaaagaaaaaagggttagtacacataaattgggaattaatgtctattgattctttt 120
121 atctttct 180
181 tttgtctctccacatcactacaaatctctctctctctctctctctctctctctctctctct 240
61 M E 80
241 aatatgggaactagagtgatagagccattgataatggggagagtggtaggagatgttctt 300
81 N M G T R V I E P L I M G R V V G D V L 100
301 gatttcttccactccaacaactaagatgaatgtagttataacaagaagcaagtctccaat 360
101 D F F T P T T K M N V S Y N K K Q V S N 120
361 ggccatgagctcttttct 420
121 G H E L F P S S V S S K P R V E I H G G 140
421 gatctcagatccttct 480
141 D L R S F F T L 160
481 TGGTTTTATTTATATTGTGCCCCAAAAAACCATATAAAAAGTCTCACTTCCTTTTCCTCT 540
541 TACAAGTTTTCCATTTCTAACTCAATAATCTTATAAACTTGTAAGCTTTAGTTTTATCAT 600
601 TCCTTTTTCCAGTCTTTTTTTTTTAATGGTAAACTCAACCGAAATGCAAAACAGgtgat 660
201 V M 220
661 gatagaccagatgttccaggctcctagtgacccctttctaaaagaacacctgcactgGTA 720
221 I D P D V P G P S D P F L K E H L H W 240
721 CGTTTAATTTATTTATTCTTTCTTTTCATTTTGGGCCCATATTCCATATACATTGCATT 780
781 AAATCATTTGTTTATAACCCTAATAAAGTTTTTTTTTGGGTGTAAGTTATATACATTTGAG 840
841 TTGGTCAAAGATCTCCATCGCCATGAGTTCTCAGAACTTTTTCTGTAAAGTAATAATATT 900
901 AGTATTGTTGAATGTTTCAATAGgagtcgttacaaacattcccggcacaacagatgctacg 960
301 I V T N I P G T T D A T 320
961 ttttgGTAAGGCCTCTTCATGAATCTTGTAATTTAAATACTTATACATATATCATGTTATA 1020
321 F G 340
1021 TAGAAATAAAAATATTTGCATTGTAATATAGgcaaagagggtggtagctatgaattgcc 1080

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Figure 6(b) Continued

341
K E V V S Y E L P 360

1081 aggccaaagcatagggatacataggtttgtgtttgttctgttcaggcagaagcaaagacgt
361 R P S I G I H R F V F V L F R Q K Q R R 1140
380

1141 gttatcttttcttaatatcccttcgagagatcacttcaacactcgtaaatttgcggtcgag
381 V I F P N I P S R D H F N T R K F A V E 1200
400

1201 tatgatcttgggtctccctgtcggggcggtcttctttaaagcacaagagaaaacgctgca
401 Y D L G L P V A A V F F N A Q R E T A A 1260
420

1261 cgcaaacgctagtttcatgattgtcataaaactgcaaaaatgaaagaagaaaatttgcattg
421 R K R * 1320
440

1321 taatctcatgtttatttgtgttctgaatttccgtactctgaataaaaactgccaaagatg 1380

1381 agttgaatccgAAATATCAATTGAGTTTACAGAAGTATTGATAACGATCT 1430

Figure 7(a)

Concen Pd71	a a g c a a c a t c	a a a a a c a g c a	t c a t a a a t c c	t t t t a c t t t t	g t t g c a t t t t	t a t c a t c t t a	60'
Concen Pd71	a t t a a g c a t t	c t t c t c a t a	t a a t a t a g t t	a t g g c a g c a a	a a g t t t c a t c	g g a c c g c t a	120
Concen Pd71	c g c g c c g a a	g t t a a c a a a	g a a a t a g a a g	a t c a t g g g a a	c t a g a g t g a t	a a a g o c a t t g	63
Concen Pd71	g t g a t a g g g a	g a g t c a t c g g	a g a c g t t g t t	g a t t c a t t c a	c t c a a c t g t	t a a a t e g t c t	180
Concen Pd71	a t e a t g g g g a	a g t g g t a g g	a a a t g t t c t t	g a t t c t c t c a	c t c a a c a a c	t a a g a t g a t	123
Concen Pd71	g t e a t t e t a c a	a t c c a a c a a	t t c c a t c a a g	c a t g t c t a c a	a t g g g c a t g a	g c t c t t c t c t	240
Concen Pd71	g t e a t t e t a c a	a t c c a a c a a	t t c c a t c a a g	c a t g t c t a c a	a t g g g c a t g a	g c t c t t c t c t	240
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	300
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	231
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	360
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	360
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	420
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	351
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	480
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	411
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	540
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	455
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	600
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	525
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	550
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	550
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	720
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	537
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	780
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	680
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	840
Concen Pd71	t c g c g t g t t a	c e t c t a c a c c	t a g g g t t g a g	g t t a t g g t g g	g t g a t a b g a g	a t c t t t c t c t	680

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Figure 7(a) Continued

Concen	a g a t g g a g a a	a t g g g a a a t t	g t g a t g t c c a	c t t g t t a t a a	a t t g a t g t a a	t t a a t t t c t a	900
Pd71	680
Concen	t g a t a t a t a a	t t t g g a a g t t	g t g t t g t g c g t g				
Pd71				

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Figure 7(b)

Cen	aagcaacatc	aaaaacagca	tcataaatcc	tttactttt	gttgcatttt	tatcatcttt	60
Arab	3
Rice291a	0
Rice291b	0
Rice1946	0
Cen	attaaagcatt	ctctctccctb	taattctagtt	atgggcaaggaa	aagttttcatc	ggaccctggcc	120
Arab	cgcgctccgaa	gtctaacabaa	gaaatctgda	atcatgggaa	ctagagtgat	agngccatcg	63
Rice291a	0
Rice291b	0
Rice1946	0
Cen	gtgattggga	gggttctcgg	agaggttggc	gattctctctb	ctctcaactgc	ttaaattctgc	180
Arab	atattggggga	gggttctcgg	agaggttggc	gattctctctb	ctctcaactgc	ttaaattctgc	125
Rice291a	0
Rice291b	0
Rice1946	0
Cen	gtctatcttaca	atctccacaaa	ttccatctgag	catggtctaca	atgggccattga	gtctctttccc	240
Arab	gtctatcttaca	atctccacaaa	ttccatctgag	catggtctaca	atgggccattga	gtctctttccc	171
Rice291a	0
Rice291b	0
Rice1946	0
Cen	tccgctgctt	ccctctacacc	tcagggttgag	gtctcatgggtg	gtggtctctcga	atcctctctcc	300
Arab	tccgctgctt	ccctctacacc	tcagggttgag	gtctcatgggtg	gtggtctctcga	atcctctctcc	231
Rice291a	1
Rice291b	0
Rice1946	0
Cen	actctgatat	tgaacagaccc	tgatgttctct	gggtccctagtg	atcccatacct	gaaggagagac	360
Arab	actctgatat	tgaacagaccc	tgatgttctct	gggtccctagtg	atcccatacct	gaaggagagac	291
Rice291a	tttcaggtta	tgaacagaccc	tgatgttctct	gggtccctagtg	atcccatacct	gaaggagagac	61
Rice291b	0
Rice1946	0
Cen	tggactgga	ttagtcaacaa	tatcccaggga	acaaactgatt	ctcccctctcc	caaggaggtc	420
Arab	tggactgga	ttagtcaacaa	tatcccaggga	acaaactgatt	ctcccctctcc	caaggaggtc	351
Rice291a	cttcattggg	ctgtcaacaa	catctccaggc	acaaactgatt	ctcccctctcc	caaggaggtc	20
Rice291b	ttacgcagga	ttgttagtga	tatacctggg	acaaactgatt	ctcccctctcc	caaggaggtc	53
Rice1946	17
Cen	gtggagcttatg	agatgcacaa	gcccgaataca	ggggtcccaaa	gggtctgtatc	tctctctgttc	480
Arab	gtggagcttatg	agatgcacaa	gcccgaataca	ggggtcccaaa	gggtctgtatc	tctctctgttc	411
Rice291a	gtggagcttatg	agatgcacaa	gcccgaataca	ggggtcccaaa	gggtctgtatc	tctctctgttc	20
Rice291b	53
Rice1946	77

[illegible]

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Figure 7(c)

Cen	.. HAAKVSSD	PLVIGRVIGD	VVDHETSSVVK	HSVIYHNNNS	IRHVYHGHEL	FPSAVISTPR	58
Arab	HENMGTRVIE	PLIMGRUVGD	VLDPETPTTK	HNVSYNK	KOVSHGHEL	FPSVSSKPR	56
Rice2918a	0
Rice2918b	0
Rice1946	0
Cen	VEVHGGDMRS	FETLMTDPP	VPGPSDPYLR	EHLHUIVTDI	PGTTDSSEFGK	EVVSXEHPRP	118
Arab	VEIHGGDLRS	FETLVHIDPD	VPGPSDPYLR	EHLHUIVTNI	PGTTDATEGK	EVVSXELPRP	116
Rice2918a	21
Rice2918b	14
Rice1946	11
Cen	NIGIHRFVFE	LEKOKKRGQA	HLSEPPVVCGRD	GFNTRKFTOE	NEGLPVAAV	FENCORETAA	178
Arab	SIGIHRFVEV	LEFONOR	VIFFNIIPSRD	HFNTRKFAVE	YELGLPVAAV	FENAORETAA	174
Rice2918a	21
Rice2918b	14
Rice1946	SIGIHRFVEV	LEKOKRRQAAV	UVPS	HFNTRQFAEE	NEGLPVAAV	FENAORETAA	68
Cen	RRR101
Arab	RKR177
Rice2918a
Rice2918b
Rice1946	RRR71

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Fig.8 (a).

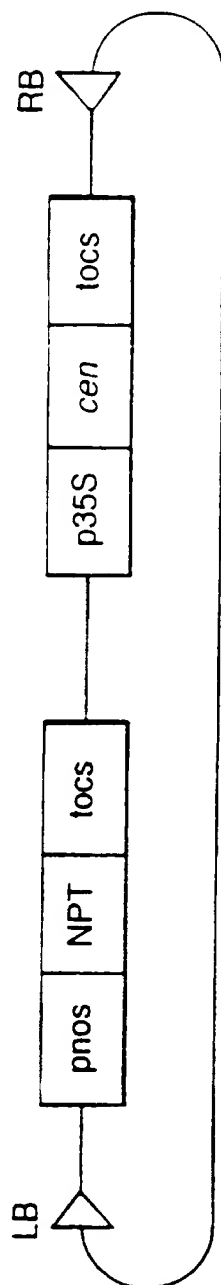
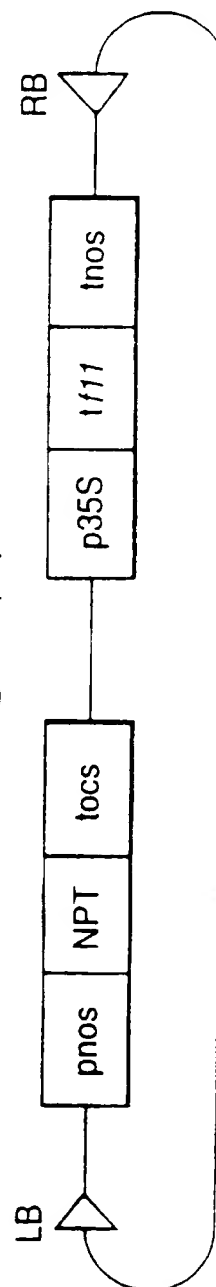


Fig.8 (b).



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 96/02276

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/29 C12N15/82 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL SEQUENCE DATABASE. REL. 42. 4-FEB-1995. ACCESSION NO. T44654, XP002024908 NEWMAN, T., ET AL.: "7917 Arabidopsis thaliana cDNA clone 129D7T7" see sequence	3-6,8,9, 11,14, 15,22,23
P,X	--- NATURE (LONDON) 379 (6568). 1996. 791-797. , XP002024909 BRADLEY D ET AL: "Control of inflorescence architecture in Antirrhinum." see the whole document --- -/-	1-10,18, 22,23

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- '&' document member of the same patent family

Date of the actual completion of the international search

10 February 1997

Date of mailing of the international search report

20.02.97

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Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No.
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DEVELOPMENT (CAMBRIDGE) 0 (SUPPL.). 1994. 107-116., XP000617343 COEN E S ET AL: "Evolution of flowers and inflorescences." see the whole document ---	1-35
A	THE PLANT CELL, vol. 3, 1991, pages 877-892, XP002024910 SHANNON, S., ET AL.: "A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development" see the whole document ---	11-15
A	THE PLANT CELL, vol. 5, no. 6, June 1993, pages 639-655, XP002024911 SHANNON, S., ET AL.: "Genetic interactions that regulate inflorescence development in Arabidopsis" see the whole document ---	11-15
A	TRENDS IN BIOTECHNOLOGY, vol. 13, no. 9, September 1995, pages 350-355, XP002024912 MOL, J.N.M., ET AL.: "Floriculture: genetic engineering of commercial traits" see page 353, left-hand column, paragraph 2 - page 354, left-hand column, line 1 ---	1-35
A	PLANT MOLECULAR BIOLOGY, vol. 25, 1994, pages 335-337, XP002024913 AN, G., ET AL.: "Regulatory genes controlling flowering time or floral organ development" see the whole document ---	1-35
A	TRENDS IN BIOTECHNOLOGY, vol. 9, January 1991, pages 31-37, XP002019122 BALCELLS, L., ET AL.: "Transposons as tools for the isolation of plant genes" see page 34 - page 35 ---	1-15
A	CELL, vol. 80, 24 March 1995, pages 847-857, XP002004926 PUTTERILL J ET AL: "THE CONSTANS GENE OF ARABIDOPSIS PROMOTES FLOWERING AND ENCODES A PROTEIN SHOWING SIMILARITIES TO ZINC FINGER TRANSCRIPTION FACTORS" ---	30-34

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 96/02276

(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>THE PLANT CELL, vol. 3, 1991, pages 359-370, XP002024914 MEDFORD, J.I., ET AL.: "Molecular cloning and characterization of genes expressed in shoot apical meristems" see the whole document -----</p>	35

